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Bioreactor Based Bone Tissue Engineering: Influence of Wall Collision on Osteoblast Cultured on Polymeric Microcarrier Scaffolds in Rotating Bioreactors

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ABSTRACT

Rotating bioreactors have been used to overcome the limitations of passive nutrient diffusion in three-dimensional (3D) constructs for tissue engineering of bone. It is hypothesized that conventional scaffolds undergo repeated wall collisions in rotating bioreactors, which may disrupt bone tissue formation. In this study, we investigated the effects of wall collision on osteoblastic cells cultured on a microsphere based scaffold of varying densities in comparison to water. The conventional heavier than water (HTW; density > 1 g/cm^3) scaffolds were fabricated by sintering HTW microspheres of 85:15 poly (lactide-*co*-glycolide) (PLAGA), and mixed scaffolds were designed by mixing lighter than water (LTW; density < 1 g/cm^3) and HTW microspheres of PLAGA. We quantified average velocities of the two types of scaffolds using a particle tracking system, and no significant difference in average velocities was observed between the two types of scaffolds. However, HTW scaffolds have frequent wall collision and mixed scaffolds can avoid wall collision in bioreactors. When human Saos-2 osteoblast like cells were cultured on the scaffolds in bioreactors for 16 days, bone cell proliferation and cell differentiation on HTW scaffolds were significantly inhibited as compared to those cultured on mixed scaffolds in rotating bioreactors. These results indicate that collision between scaffolds and bioreactor wall is a confounding factor in osteoblastic cell proliferation and differentiation. These studies provide a foundation for development of 3D scaffolds for tissue engineering of bone in rotating bioreactors.

INTRODUCTION

Due to the limitations associated with biological and synthetic bone grafts, tissue-engineering approaches have been widely adopted for the development of bone substitutes. Studies have suggested that the limited diffusion in static culture environments may constrain tissue ingrowth in tissue-engineered constructs. To overcome the limitations associated with static culturing systems, considerable interest has been generated in tissue engineering by using the high aspect ratio vessel (HARV) rotating bioreactor. It is hypothesized that repeated collisions between scaffolds and bioreactor wall might disrupt bone tissue formation in the rotating bioreactors [1-3]. However, no study has explicitly demonstrated the role of wall collision in rotating bioreactors on osteoblastic cell proliferation and differentiation. In previous studies, we have described the development of novel poly(lactide-co-glycolide) (PLAGA) microsphere based mixed scaffolds that can avoid wall collision in rotating bioreactors [4-6]. As a result, differentiation and mineralization of human osteoblastic cells (Saos-2) and rat calvarial cells seeded on these scaffolds were significantly enhanced [4-8]. The trajectories of these scaffolds could be adjusted by HTW and LTW microcarriers in different ratios. In this study, we seek to test the effects of wall collision on cell proliferation and differentiation of human Saos-2 osteoblastic cells cultured on conventional HTW and mixed PLAGA microcarrier scaffolds with similar average velocities in rotating bioreactors.

MATERIALS AND METHODS

Scaffold preparation: LTW and HTW biodegradable polymeric microspheres were fabricated using PLAGA copolymer (Alkermes Medisorb®, Wilmington, OH) in an 85:15 ratio as described previously [4-6]. The polymer is amorphous, and has an inherent viscosity of 0.66-0.80 dl/g and glass transition temperature of 50-55°C. Briefly, PLAGA was dissolved in methylene chloride (Aldrich, Milwaukee, WI) at 30% (wt/v), and 10% (v/v) of distilled water was added into the solution to generate bubbles. The solution was slowly poured into a 0.1% (w/v) polyvinyl alcohol (PVA; MW=30,000-70,000 D, Sigma, St. Louis, MO) solution stirring at 1500 rpm, and the solvent was allowed to evaporate over night at room temperature. The LTW (present at surface of the solution) and HTW (present at bottom of the solution) microspheres were collected separately, and washed extensively with distilled water to remove the remaining solvent and PVA. The microspheres were freeze-dried using a lyophilizer (Labconco, Kansas City, MO) overnight. The LTW and HTW biodegradable polymeric microspheres were sieved into different sizes, and microspheres with diameters from 425-500 μm were used to fabricate the scaffolds. The 3-D microsphere scaffolds were fabricated into 4 mm x 2.5 mm (diameter x height) cylindrical scaffolds of varying density using sintered microsphere methods described previously [4-6]. Briefly, the HTW scaffolds were fabricated by sintering HTW polymeric microspheres of PLAGA at 80°C for 3 hours. The mixed microsphere scaffolds were fabricated by sintering the HTW and LTW microspheres in the ratios (wt/wt) of 60:40 at 80°C for 3 hours.

Scaffold motion tracking: The movement of scaffolds was tracked using a real time microcapsule visualization unit, which comprises a CCD camera (Cohu, Inc. San Diego, CA) that is rotated in synchrony with a rotating bioreactor [9]. Scaffold motion was recorded digitally with a video cassette recorder (Sony SVO-9500, MD) and analyzed using Image Pro (Phase 3 Imaging). Using the tracking apparatus, scaffolds were visualized in a bioreactor rotating at 36-rpm rotation, and the average velocity values of the scaffolds were calculated by dividing the distance traveled by the scaffold by the time interval between frames.

Cell culture: HTW and mixed scaffolds were sterilized under UV light for 45 minutes, followed by 70% ethanol for 20 minutes and washed with phosphate buffered saline (PBS; Cibco BRL, Grand Island, NY) twice for 15 minutes. Human saos-2 cells were seeded statically onto microcarrier scaffolds at a density of 5 x 10^4 cells/scaffold. After 24 hr (day 0), scaffolds were transferred to 50 ml HARV vessels (Synthecon, Huston, TX) either maintained statically as controls or rotated at 54 rpm as rotating bioreactors. The cells were cultured in Hank's F-12 media supplemented with 15% fetal bovine serum (FBS; Gibco, BRL), 1% penicillin/ strepmycin (P/S; Gibco, BRL), 1% glucose (Sigma), and 1% ß-glycerophosphate (Sigma) at 37°C and 5% CO_2. The media were changed every 4 days, and the cultures were maintained for 16 days. At days 4, 8 and 16, scaffolds were removed and characterized for cell proliferation and differentiation.

Cell proliferation: At days 4, 8, and 16, cell proliferation was analyzed by utilizing cell proliferation assay solution (CellTiter; Promega). This assay is based on the ability of live cells to reduce a tetrazolium-based compound to a purplish formazan product. Briefly, scaffolds were washed with PBS, transferred into new petri dishes containing 1 ml culture media and 200 µl of cell proliferation assay solution and incubated for 2 h at 37°C. The scaffolds were washed extensively by pipetting up and down repeatedly to allow total color release. The absorbance of the supernatant was read using a spectrophotometer at 490 nm.

Cell differentiation: Cell differentiation was analyzed by measuring alkaline phosphatase activity. The colorimetric method was based on the conversion of p-nitrophenyl phosphate into 1 ml of p-nitrophenol, in the presence of alkaline phosphatase. Briefly, the scaffolds were put in 1% triton X-100 at room temperature for 1 hour. A volume of 100 µl sample solution was added to 1 ml of p-nitrophenyl phosphate solution (16 mM) (Dignostic Kit 245, Sigma) at 37°C for 15 minutes. The production of p-nitrophenol was determined by measuring the absorbance using a micro-plate reader (Shimadzu, Maryland) at 405 nm.

Statistical Analysis: A two-tailed student t-test was used for comparing the results between the static and rotating culturing groups. A p value less than 0.05 was considered to be statistically significant.

RESULTS

Tracking of Scaffolds

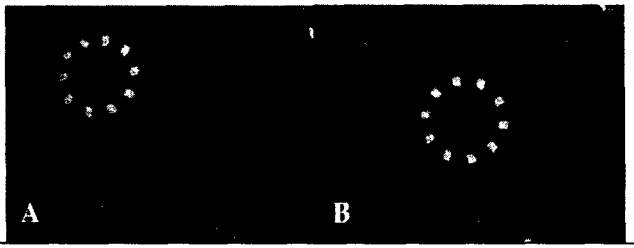

Figure 1: Trajectories of scaffolds in rotating bioreactors. A) HTW scaffolds; B) Mixed scaffolds (HTW:LTW, 60:40).

The scaffold trajectories of HTW and mixed scaffolds are shown in Figure 1. HTW scaffolds undergo frequent wall collision (Figure 1A). The aggregate densities of the mixed scaffolds (HTW:LTW, 60:40) are less than the surrounding medium, and the buoyant forces drag the scaffolds toward the center of the bioreactor vessel and keep the scaffolds from collisions with the bioreactor wall (Figure 1B). Based on the motion trajectories of scaffolds and the rotating speed of bioreactor, we measured the average velocities of scaffolds in rotating bioreactors by dividing the distance traveled by the scaffolds in a full circle by the time interval. The average velocity values of the HTW and mixed scaffolds are 47.4 ± 4.9 mm/s and 52.3 ± 7.4 mm/s respectively. There is no significant difference between the average velocities of the conventional HTW scaffolds and mixed scaffolds.

Cell proliferation

As shown in Figure 2, the absorbance for cell proliferation changed dramatically at different conditions after 4, 8 and 16 days of culture. The absorbance for osteoblastic cell proliferation on HTW scaffolds in rotating bioreactors significantly decreased as compared to those of static controls, and the absorbance for osteoblastic cell proliferation on the mixed scaffolds in rotating bioreactors significantly increased as compared to those of HTW scaffolds in rotating bioreactors. Therefore, osteoblastic cell proliferation on HTW scaffolds in rotating bioreactors were significantly inhibited as compared to those of static controls, and osteoblastic cell differentiation on the mixed scaffolds in rotating bioreactors were significantly enhanced as compared to those of HTW scaffolds in rotating bioreactors.

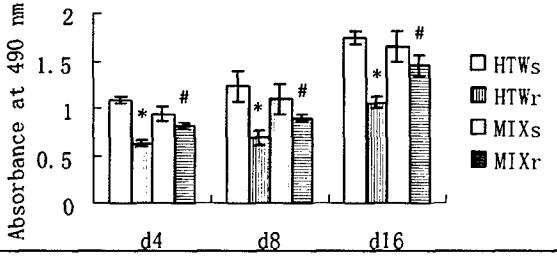

Figure 2. Cell proliferation. * indicates significantly lower ($p < 0.05$) absorbance as compared to that of static control; # indicates significantly higher ($p < 0.05$) absorbance as compared to that of HTW scaffolds cultured dynamically.

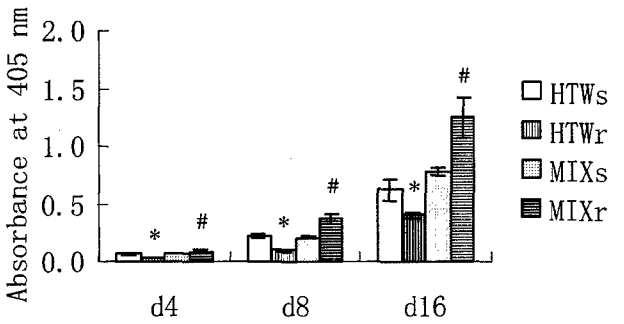

Figure 3. Alkaline phosphatase activity. * indicates significantly lower ($p < 0.05$) absorbance as compared to that of static control; # indicates significantly higher ($p < 0.05$) absorbance as compared to that of HTW scaffolds cultured dynamically.

Cell differentiation

As shown in Figure 3, the absorbance from alkaline phosphatase assay changed dramatically at different conditions. Compared to static conditions, the expression of alkaline phosphatase in cells cultured on HTW scaffolds in rotating bioreactors significantly decreased ($p < 0.05$) at days 4, 8 and 16. The absorbance from alkaline phosphatase activity for cells on mixed scaffolds in rotating bioreactors significantly increased ($p < 0.05$) as compared to those of HTW scaffolds in rotating bioreactors. Therefore, osteoblastic cell differentiation on HTW scaffolds in rotating bioreactors were significantly inhibited as compared to those of static controls, but osteoblastic cell differentiation on the mixed scaffolds in rotating bioreactors were significantly enhanced as compared to those of HTW scaffolds in rotating bioreactors.

DISCUSSION

Studies have shown that the conventional scaffolds (scaffolds with greater aggregate density than the surrounding medium) undergo repeated collisions with the bioreactor wall, and repeated wall collision inside the rotating bioreactor may lead to cell damage and limit tissue synthesis [1-3]. However, as different cells, scaffolds and experimental conditions were utilized, the role of wall collision on osteoblastic cells cultured on 3D scaffolds in rotating bioreactors were complicated by many factors. In the current study, we demonstrated that osteoblastic cell proliferation and differentiation on HTW scaffolds in rotating bioreactors were significantly inhibited, and osteoblastic cell proliferation and differentiation on mixed scaffolds in rotating bioreactors could be improved by adjusting motion trajectories to avoid wall collision. As the average velocities of the two types of scaffolds in rotating bioreactors are similar, the difference in osteoblastic cell proliferation and differentiation between HTW and mixed scaffolds in rotating bioreactors is associated with wall collision. The specific mechanism of collision-induced bone tissue synthesis has yet to be elucidated, and our current study is helpful to further clarify the role of wall collision in rotating bioreactors for bone cell function within 3D scaffolds.

CONCLUSIONS

These studies suggest that osteoblastic cell proliferation and differentiation on HTW and mixed scaffolds with similar average velocities in rotating bioreactors are significantly different. While osteoblastic cell proliferation and differentiation on HTW scaffolds in rotating bioreactors were significantly inhibited due to collision between HTW scaffolds and bioreactor wall, osteoblastic cell proliferation and differentiation on mixed scaffolds in rotating bioreactors were improved by avoiding wall collision. These studies provide a basis for the optimization of scaffold design in rotating bioreactors for bone tissue engineering.

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